IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	: 10/779,746
Applicant	: Sheldon B. GREER
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Examiner	: James D. Anderson
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Customer No.	: 06449
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DECLARATION

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Sheldon Greer, declare as follows.

1. I am the inventor of the above-captioned U.S. patent application. I am a professor of Microbiology, Immunology, Biochemistry, Molecular Biology and Radiation Oncology at the University of Miami School of Medicine. I have researched radiosensitization of tumors over twenty years.

2. I understand that the Examiner rejected the above-captioned U.S. patent application based on the combination of Russell (Cancer Research 46:2883-87 (1986)), and Nagatake (Cancer Research 56:1886-91 (1996)). I understand that the Examiner also rejected the above-captioned U.S. patent application based on the combination of Greer (WO85/01871), Nagatake and Shepherd (Cancer 70:2250-54 (1992)).

3. Prior to the filing of the present application, one skilled in the art (including me and many respected oncologists) believed that, in radiation cancer therapy, 5-

chloro-2'-deoxycytidine ("CldC") and tetrahydrouridine ("H4U") alone were not clinically acceptable or not clinically useful, and that CldC and H4U must be used together with a pretreatment agent such as N-(phsphonoacetyl)-L-aspartate ("PALA") and (5-fluoro-2'-deoxycytidine ("FdC") to achieve a clinically meaningful radiosensitization. Such prior art understanding is clearly described in the present application, page 1, the third full paragraph ("[PALA, FdC, H4U and CldC] protocol was unamenable to modifications"). In fact, in my opinion, the Russell and Greer references, which the Examiner relied on to find CldC and H4U used for radiosensitization, also show such prior art understanding.

4. The Greer reference, which is my old patent application, does state that CldC and H4U sensitize tumors to radiation, as the Examiner notes. I claimed a method of sensitizing susceptible neoplastic tissue to radiation using CldC, for example, in claim 1 and using CldC and H4U, for example, in claim 4. However, when I claimed a method of treating neoplastic tissue in a patient (claims 11-17), I required a pretreatment step employing a compound such as FdC, FdU and PALA (see claim 12) prior to administering CldC and H4U. As it is stated at page 13, lines 31-36 of the Greer reference, I had the opinion that "lowering both PALA and CldC concentrations results in significant loss of radiosensitization..." and PALA was as essential as CldC for radiation cancer therapy. In my opinion, a person having ordinary skill in the art would agree with my previous conclusion if he read through the Greer reference, without the knowledge of the present application, since I emphasized the importance of the

pretreatment step before the administration of CldC and H4U many times in the Greer reference, including at pages 11-13 and 16-19.

5. In my opinion, the Russell reference is not much different from the Greer reference in that the Russell reference teaches that, in radiation therapy, CldC and H4U without PALA and FdC were not clinically acceptable or not clinically useful since the Russell references teaches that "[a] trial of [H4U] provided the simplest approach to potentially maximizing [CldC] effects but yielded no differences beyond that obtainable with [CldC] alone." Page 2885, the third full paragraph. CldC has not been approved for clinical use by the FDA. Further, scientists did not achieve a clinically meaningful radiation therapy regimen with only CldC at the time of filing, as evidenced by all the publications that attempted to develop a radiation therapy regimen employing a combination of CldC with another compound. Russell further reports that "under our conditions of drug incorporation, we found no advantage of [CldC] over BrdUrd in the *in vivo* experiments with this particular tumor: equimolar infused doses produced less radiosensitzation by [CldC] than by BrdUrd." Page 2886, the second full paragraph. Therefore, in view of the fact that CldC at the time of filing did not achieve a clinically meaningful radiation *in vivo* radiosensitization level, the Russell reference, reporting that CldC has no advantage over BrdUrd, and CldC and H4U has no advantage over CldC, is basically telling a person having ordinary skill in the art that CldC and H4U is a failed attempt to achieve a clinically meaningful radiation therapy regimen, and therefore, further investigation should be focused on BrdUrd.

6. In fact, as a result of the Russell reference, following the publication of the Greer reference in 1985, and the Russell reference in 1986, prominent oncologists Dr. Kinsella and Dr. Cheng reported in 1994, referring to the Russell reference, that "investigators have not found an advantage to ICdR compared to IUdR as an *in vivo* tumor radiosensitizer ... [d]ue to these conflicting experimental data, the cytidine derivatives have not been tested in recent clinical trials." Kinsella, Cancer Research 54:2695-700 (1994), page 2695, the third full paragraph. It must be noted that Kinsella and Cheng mistakenly used the term "ICdR" to mean "CldC" as evidenced from the fact that the references 14-16 in the Kinsella reference are all directed to CldC. Particularly, the Russell reference discusses only CldC, and does not mention ICdR at all.

7. On the other hand, the PALA, FdC, H4U and CldC protocol ("Standard Protocol") faced serious criticisms during the period of 1991-1998. PALA and FdU were criticized for their high cytotoxicity. The fact that four variables (PALA, FdC, H4U and CldC) were involved was criticized, too, and the complexity of the dosing schedule and drug interactions were also criticized.

8. However, the efforts to simplify the Standard Protocol were not successful prior to the filing of the present application. For example, I published in 1992 that "[t]he lack of tumor regrowth delay with high doses of CldC + H_4U or IdU when used alone and the relative ineffectiveness of FdU in these studies suggest that the Standard Protocol is unammendable." Greer, Int. J. Radiation Oncology Biol. Phys. 22:505-10 (1992), page 509, the paragraph bridging the left and right columns. I also published in

1995 that "[t]he following conditions failed to result in tumor regrowth delay or cures: CldC + H_4U administered once per day Tuesday to Friday without other modulators; FdC +H_4U ...; and [Standard Protocol] with no PALA ..." Greer, Int. J. Radiation Oncology Biol. Phys. 32:1059-69 (1995). The 1995 Greer reference discloses many additional approaches that I took to simplify the Standard Protocols, all of which unfortunately were not successful.

9. Therefore, it is my opinion that a person having ordinary skill in the art would not have had a reasonable expectation that the radiation therapy regimen employing CldC and H4U and excluding PALA, FdC and FdU would be clinically acceptable or clinically useful because many prior art publications, including the Greer and Russell references cited by the Examiner, concluded that the Standard Protocol (a radiation therapy regimen employing CldC, H4U, FdC and PALA) was unammendable based on experimental data, or taught that CldC and H4U alone failed to achieve a clinically meaningful level of radiosensitization. In fact, as noted above, Dr. Kinsella and Dr. Cheng in their 1994 coauthored publication state that "cytidine derivatives have not been tested in recent clinical trials" because of the controversy over the effectiveness of CldC, relying on the Russell reference, which criticized CldC having no advantage over the first generation radiosensitizer BrdUrd.

10. However, against that bellef, which many scientists in the art possessed as shown above, that the radiation therapy regimen employing CldC and H4U without PALA and FdC would not be able to achieve a clinically meaningful level of

radiosensitization of a cancer patient, I finally discovered through my persistent efforts that the radiation therapy regimen employing CldC and H4U without PALA and FdC achieves a clinically meaningful radiosensitization in cancer patients. By having nude mice with transplanted human tumors undergo fragmented (i.e., chronic) radiation as well as chronic drug administration employing CldC and H4U without PALA and FdC, I finally achieved a clinically meaningful radiosensitization using CldC and H4U without PALA and FdC, i.e., the simplification of the Standard Protocol, which was sought after for years. This breakthrough was possible because I used nude mice with transplanted human tumors and exposed the nude mice to fragmented rather than acute radiation as well as chronic drug administration, unlike the prior art references using human tumor cell culture, or non-human tumors transplanted in mice, or acute (i.e. one dose or unfragmented) radiation. For example, the Russell reference applied one acute dose of radiation to single cells of the mouse tumor after sacrificing the mice and trypsinizing the tumor to obtain separate cells. Further, surprisingly by omitting PALA and FdC, I was able to increase the dose of CldC without increased toxicity in my radiation therapy regimen employing CldC and H4U without PALA and FdC and treat the model system of a human cancer patient (a nude mouse with transplanted human prostate tumor) better or at least as well as the Standard Protocol radiation therapy regimen employing all of CldC, H4U, PALA and FdC.

11. The data that I obtained from nude mice with transplanted human prostate tumors show that the radiation therapy regimen employing CldC and H4U without PALA

and FdC treats the model system of a human cancer patient (a nude mouse with transplanted human prostate tumor) better or at least as well as the Standard Protocol radiation therapy regimen employing all of CldC, H4U, PALA and FdC. In the present application, Table 2, compare the fraction of cures for the Standard Protocol ("StP$^{//}$ + X-ray") and radiation employing CldC and H4U without PALA and FdC ("StP c (|) CldC$^{//,d}$ + X-ray"). See also Figures 1b and 1c. This result was never achieved prior to the present application, and in fact, as noted above, many oncologists (including me) believed that the radiation therapy regimen employing CldC and H4U without PALA and FdC would not be able to achieve such a result.

12. Further, in my opinion, the above result obtained from the human cancer patient model system (nude mice with transplanted human tumors) is applicable to other types of cancers, for example, cancers due to gene silencing caused by hypermethylation of nucleic acids as explained at pages 13, line 9-page 14, line 18 of the present application, and cancers with elevated enzymatic activity of deoxycytidine kinase ("dCK") and dCMP deaminase ("dCMPD") in tumor cells compared with normal cells, as explained at page 13, lines 2-9 and page 27, lines 11+ of the present application.

13. Based on enzymatic studies, I attest that

approximately 35% of patients suffering from squamous cell carcinoma of the lung have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 25% of patients suffering from adenocarcinoma of the lung have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 67% of patients suffering from adenocarcinoma of the rectum have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 73% of patients suffering from infiltrating ductal carcinoma of the breast have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 53% of patients suffering from the brain tumor have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 55% of patients suffering from the head and neck tumor have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells;

approximately 22% of patients suffering from the pancreas tumor have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells; and

approximately 57% of patients suffering from the cervix tumor have 1.5 fold higher dCK activity in the tumor cells compared with the normal cells and 2 fold higher dCMPD activity in the tumor cells compared with the normal cells.

14. I also tested with several human cancer patient model systems (nude mice with transplanted human tumors). As noted above in paragraph 11, the present application has prostate cancer patient data. As shown in my 2001 publication (Greer, Int. J. Radiation Oncology Biol. Phys. 51:791-806, copy attached), I have another set of prostate cancer patient data (Figures 2 and 5). Further, as shown in my NIH grant application (relevant pages attached), I have head and neck cancer patient data (Figures 6 and 7). These model system data show that a radiation therapy regimen employing CldC and H4U and excluding PALA, FdC and FdU in fact treats cancer patients. In addition, as shown in an actual human cancer (head and neck) patient data (attached), after following a radiation therapy regimen employing CldC and H4U and excluding PALA, FdC and FdU, CldU derived from CldC was incorporated in DNA in greater than 2 --fold more cells of the tumor compared with the number of normal mucosal cells, indicating Selectivity, so that tumor cells become much more sensitive to radiation.

15. Therefore, in my opinion, the radiation therapy regimen employing CldC and H4U without PALA and FdC are clinically effective and acceptable to treat human cancer patients, particulary cancer patients with increased enzymatic activity of dCK

and dCMPD in the tumor cells compared with normal cells, and particularly to treat lung, rectum, breast, head & neck, brain, pancreas, prostate and cervix cancers.

16. I state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Sheldon Greer

Sheldon Greer

2/26/07

Date

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